# PUBLICATIONS WITH ABSTRACTS OF THE PHARMACOLOGY LABORATORY

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A limited number of the reprints of the publications are available. Those not available are marked with an asterisk (*).

Studies of phenothiazine. X. Further observations on oxidation of phenothiazine. F. DeEds. Proc. Soc. Expt. Biol. and Med. 45(2):632-634, Nov., 1940. Developments on the use of phenothiazine as a fungicide pointed to the importance of the observation that in the dry state phenothiazine oxidizes, a response which having been repeatedly confirmed, was deemed worthy of a report. The experiments reported here suggest that the distribution of phenothiazine over the extensive surface presented by bentonite permits intimate contact with atmospheric oxygen, thereby facilitating oxidation, an action which may be further aided by the alkalinity of the hydrated lime.

Studies on phenothiazine. IX. The biliary excretion and anthelmintic action of thionol. F. DeEds and J. O. Thomas. Jour. Parasitology 27(2):143-151. April, 1941. Phenothiazine is one of the most effective, and certainly the most versatile, of the organic compounds developed by the U. S. Department of Agriculture. In addition to being a promising insecticide, phenothiazine has been shown to be useful as a ruinary antiseptic, and is now receiving considerable attention as an anthelmintic for removal of parasites from farm animals, notably sheep. The demonstration that thionol, an oxidation product of phenothiazine, is excreted in the bile may be important to our understanding of the anthelmintic action of phenothiazine.

The toxicity of fluorine in dicalcium phosphate. F. DeEds. Amer. Jour. Med. Sci. 203(5):678-692. May, 1942. Dicalcium phosphate is used as a dietary supplement during pregnancy, and for infants and children. Since fluorine is present in dicalcium phosphate as an impurity due to the sources of raw material or methods of manufacture, obstetricians and pediatricians should give consideration to the possibility of chronic fluorine poisoning. This paper reports a study of the toxicity of fluorine present in dicalcium phosphate, using the bleaching of rat incisor teeth as a criterion of injurious action. The fluorine present in dicalcium phosphate has been shown to be as physiologically active as fluorine administered as sodium fluoride. An average daily dose of 1 teaspoonful of dicalcium phosphate containing 0.27 percent fluorine represents a fluorine intake 10 times as great as the amount said to produce mottled enamel in at least some children.

*Vitamin A value of fresh and dehydrated carrots. R. H. Wilson, J. O. Thomas, and F. DeEds. Fruit Prod. Jour. 22(1):15-17. Sept., 1942. This paper is concerned with the vitamin A value of fresh and dehydrated carrots of the Chantanay variety. The parallelism between the chemical and the biological methods for evaluating vitamin A both before and after a processing technique is studied.

Studies on phenothiazine. XI. The excretion of phenothiazone. F. DeEds and J. O. Thomas. Jour. Parasitol. 28(5):363-367, Oct., 1942. Evidence is presented in this paper to show that the reversible oxidation-reduction system phenothiazone-leucophenothiazone, as well as thionol-leucothionol, occurs in the urines of rats, rabbits, and humans receiving phenothiazine. The validity of potentiometric measurements for the identification of a reversible oxidation-reduction system is discussed. The melting point of leucophenothiazone was shown to be 172-173° C. Samples of leucophenothiazone isolated from rat, rabbit, and human urines were identified by their melting points, namely, 172-173° C. and by their mixed melting points with synthetic leucophenothiazone. Leucothionol oxidizes so rapidly that it is impracticable to obtain its melting point. It is suggested that a compound isolated from the urine of a phenothiazine-dosed sheep by another worker was leucophenothiazone and not phenothiazine.

*Protein-ascorbic acid complex in carrots. F. DeEds. Food Res. 8(4):275-279, July-Aug., 1943. This paper reports that carrots, like certain other vegetables, contain protein-combined ascorbic acid. Since ascorbic acid in this form is biologically available, it must be taken into consideration in determining the vitamin C value of different foods and in comparing the bioassay and chemical methods. The degree of protection which such a combination may afford ascorbic acid during dehydration and processing of food is worthy of investigation.

Acute and subacute toxicity of pure citrinin. A. M. Ambrose and F. DeEds. Proc. Soc. Expt. Biol. and Med. 59(2):289-291, June, 1945. Citrinin is an antibiotic produced by Penicillium citrinum, and by Aspergillus sp. of the candidus group. The lower toxicity values reported by Robinson, as compared with those reported by Timonin and Rouatt and those reported here, may be due to a low rate of absorption of citrinin from a suspension in gum acacia solution. Citrinin in solution is rapidly absorbed, regardless of the mode of administration, as shown by the toxicity data. The production of tissue changes and the fact that citrinin may result in delayed deaths, up to fourteen days, would make a statement regarding an $LD_{50}$ dose misleading.

Norelac--A substitute for shellac in the preservation of smoked paper records. A. M. Ambrose and F. DeEds. Science 102(2642):179-180, Aug., 1945. This paper reports that Norelac, a thermoplastic polymer developed at the Northern Regional Research Laboratory, can be substituted for shellac with complete satisfaction. A 5-percent solution of Norelac in a mixture of isopropyl alcohol and Skelly Solvent "C" (or naphtha) makes a good protective coating for a smoked-paper record. The record dries in ten minutes with a dull finish. If less than 5 percent of Norelac is used, abrasion marks are easily produced. If 10 percent of Norelac in isopropyl alcohol and Skelly Solvent "C" is used, the record dries free from tack in ten minutes with a glossy finish.

The content and biological availability of carotene in raw and dehydrated carrots and other vegetables. R. H. Wilson, A. M. Ambrose, F. DeEds, H. J. Dutton, and G. F. Bailey. Arch. Biochem. 10(1):131-140, May, 1946. Dehydrated carrots stored at 98° F. and 120° F. in an atmosphere of $CO_2$ were changed so as to be unacceptable from an aesthetic and organoleptic point of view, but carotene content was not lowered at 120° F. as much as during storage at 98° F. or lower. That the partial destruction of the carrots at the elevated temperature created conditions unfavorable to the oxidation of

carotene is suggested. Spectrophotometric analysis and bioassay of extacts of these products agreed well. However, when the solid material was used for bioassay, less than one-third of the carotene present was available to the rat.

Some toxicological and pharmacological properties of citrinin. Anthony M. Ambrose and Floyd DeEds. J. of Pharm. & Expt. Therap. 88(2):173-186, Oct., 1946. The isolation and purification of citrinin from a natural culture medium has been described. Toxicological data and symptomatology for rats, mice, rabbits, and guinea pigs have been described in detail. In general the symptoms observed after suitable doses of citrinin, by various routes of administration, were - miosis, hyperemia, salivation, increased bronchial secretions, lacrimation, and in rabbits marked peripheral vasodilatation of the ear vessels. These effects were not completely annulled by doses of atropine used.

Studies of the action of citrinin on isolated organs revealed constriction of the bronchi, increased tone of cardiac muscle, decreased tone of the uterus, increased tone of the intestine, and increased tone of the frog gastocnemius. In dogs citrinin produced an evanescent fall in blood pressure which was associated with peripheral vasodilatation and increased peristalsis of stomach and intestine. In the atropinized or vagotomized dog a reversal of the depressor to pressor action was observed, where the muscarinic effects were abolished. From these observations and those on the rabbits' ears it appears that citrinin possesses a nicotine-like action similar to acetylcholine.

Some comparative observations on l-nicotine and myosmine. Anthony M. Ambrose and Floyd DeEds. Proc. Soc. Expt. Biol. Med. 63(2):423-424, Nov., 1946. Studies are presented on the comparative toxicity and physiological action of myosmine and l-nicotine. The acute toxicity of myosmine was about one-tenth that of l-nicotine after oral administration. Intraperitoneally myosmine was about one-sixth as toxic as l-nicotine. On isolated intestinal strips of guinea pigs, contraction by myosmine required about 200 times the concentration necessary in the case of l-nicotine. Sulfathiazole inhibited the action of both myosmine and l-nicotine on isolated intestinal strips.

Experimental studies on the use of starch as surgical dusting powder. Shannon C. Allen and Floyd DeEds. Fed. Proc. 6(1):305-306, March, 1947. Several samples of starch powders, possible substitutes for talc as surgical dusting powder, were compared (for foreign body reactions) with talc, potassium bitartrate and potassium acid saccharate. Some samples left the peritoneal cavity in a completely normal condition after an initial inflammatory reaction, while others produced numerous adhesions. Evidence indicates that starch can be used successfully as surgical dusting powder but that its safety is determined by the type of processing it has undergone.

The protective action of rutin against capillary injury. Anthony M. Ambrose and Floyd DeEds. Fed Proc. 6(1):306, March, 1947. Rutin has been reported to have beneficial actions on conditions of impaired capillary permeability or fragility. The possibility of rutin protecting against capillary injury has been tested experimentally in rabbits using the accumulation of intravenously injected trypan blue into the irritated areas as a criterion of capillary damage. Capillary injury was produced by means of chloroform, intracutaneous injection of histamine, and the application of negative pressure. These procedures were applied to the depilated ventral surfaces of albino rabbits.

After control observations on the time of accumulation of dye into chloroform-irritated areas, the rabbits received intravenously 1 ml./kg. of a 20% solution of rutin in propylene glycol.
In 22 rabbits, after the injection of rutin, the appearance of dye in newly formed chloroform wheals was delayed as much as 8 times over the control observations. Intracutaneous injection of histamine before and after rutin administration gave some indications that rutin protected against the local action of histamine. Five out of 6 rabbits to which negative pressure (30 mm. of Hg) was applied for 1 minute showed blue stained wheals immediately after release of suction before rutin injection, but none showed positive results when negative pressure was reapplied after rutin.
It is concluded that rutin protects rabbits against capillary damage under the conditions of our experiments.

The biological availability of l-ascorbyl palmitate. Anthony M. Ambrose and Floyd DeEds. Arch. of Biochemistry 12(3):375-379, March, 1947. A comparison of the biological availability of l-ascorbic acid and l-ascorbyl palmitate has been made, based upon the increase in serum "alkaline" phosphatase of scorbutic guinea pigs.
l-ascorbyl palmitate was found to have antiscorbutic activity comparable to an equivalent amount of ascorbic acid, 2.36 mg. of the ester being equivalent to mg. of l-ascorbic acid.

*The toxicity and pharmacology of rutin. R. H. Wilson, T. G. Mortarotti and F. DeEds. Fed. Proc. 6(1):385, March 1947. No acute or chronic toxicity of rutin was detected in experimental animals, with doses ranging up to 100 mg./kg., parenterally, and up to 1% of the diet for the chronic studies. Rutin prolonged the action of epinephrine on intestinal strips, with little direct action on the strips except at high dosage levels. It increased the amplitude of beat of the isolated heart, with a depression of the beat much after brief stimulation. In guinea pigs, it furnished partial protection against an $LD_{50}$ dose of histamine.

Toxicity studies on rutin. R. H. Wilson, T. G. Mortarotti and E. K. Doxtader. Proc. Soc. Exp. Biol. and Med. 64(3):324-327, March, 1947. Rutin, a flavonol glycoside with vitamin P activity, was studied for signs of acute and chronic toxicity. In rats and guinea pigs, intravenous and intraperitoneal injections of 30 to 50 mgm./kg., and intravenous injections in rabbits of 100 to 200 mgm/kg., were inocuous. The rate of growth of albino rats was not affected when the diet contained as much as 1 per cent of rutin, and after 400 days on such a diet, histological examination of the tissues showed no evidence of injury which could be definitely related to rutin administration. Organ weights of the experimental animals were normal. The length of the estrus cycle was the same in rats eating a 1 per cent rutin diet as in control animals, reproduction was as good, and the young appeared to be as healthy. On the basis of the criteria employed, rutin is non-toxic both acutely and chronically.

Some pharmacological properties of rutin. R. H. Wilson, T. G. Mortarotti and F. DeEds. J. Pharmacol. & Expt. Therap., 90(2):120-127, June, 1947. Rutin prolongs the action of epinephrine on intestinal strips, presumably due to protection of epinephrine from oxidative destruction. In somewhat larger concentrations, rutin per se sometimes has a direct relaxing action on intestinal muscle. Administration of rutin intraperitoneally to guinea pigs decreases the mortality due to an approximately $LD_{50}$ dose of histamine, if

the rutin precedes the histamine by 10 to 30 minutes. Simultaneous introduction of the two substances does not decrease the mortality, nor is there protection of the animal if the time between the two drugs is as much as 45 minutes to an hour. That the protection against histamine is indirect is shown by the absence of protection when the two drugs are injected simultaneously, and by lack of antagonism on perfused isolated bronchi and on strips of guinea pig colon. It is tentatively suggested that the protection of the animals from histamine shock is due to a slightly increased epinephrine level in the blood. Rutin, by an in vivo retardation of epinephrine destruction, would lead to an increase in epinephrine concentration. In our hands, the capillary fragility method for assaying vitamin P activity on guinea pigs was not effective. A modification of this method, or some other method that would work in all laboratories, is highly desirable.

The carcinogenic activity of 2-acetaminofluorene. II. Effects of concentration and duration of exposure. R. H. Wilson, F. DeEds and A. J. Cox, Jr. Cancer Res. 7(7):444-449, July, 1947. 2-Acetaminofluorene (AAF) had a carcinogenic action on albino rats when its concentration in the diet was as low as 0.004 per cent. A diet containing 0.001 per cent had no recognizable carcinogenic effect. A single massive oral dose was not carcinogenic. Feeding to rats of a diet containing 0.125 per cent AAF for 25 days initiated changes which showed up later as tumors. The length of time between the start of AAF feeding and the observation of tumors increased with decreasing concentrations of AAF and with decreasing times of administration of the carcinogenic agent.
Mice of three strains, fed AAF-containing diets, developed tumors resembling certain of those seen in rats. The mice were more resistant than the rats as judged by the frequency of tumors, time of tumor development, and concentration of AAF which the animals could tolerate. Mammary tumors were infrequent and epithelial tumors of the head were absent in the mice, although they were frequent in the rats.

The carcinogenic activity of 2-acetaminofluorene. III. Manner of administration, age of animals, and type of diet. R. H. Wilson, F. DeEds and A. J. Cox, Jr. Cancer Res. 7(7):450-452, July, 1947. The most effective way found so far to produce cancerous lesions in rats with 2-acetaminofluorene (AAF) is to feed the compound in the diet for a considerable time. A single, large, oral dose is ineffective. Introduction of powdered AAF into the external ear did not produce epithelial tumors of the head, or lesions in other organs which were clearly attributable to AAF. Subcutaneous injection of AAF dissolved in propylene glycol led to a few changes, some of them tumors, which were not clearly the result of AAF. The subcutaneous implantation of crystalline AAF caused minimal changes after a prolonged time.
Preliminary observations, which subsequent studies indicate may have to be accepted with caution, showed that vitamin enrichment of the diet did not change the incidence or time of development of the tumors. The age of the rats had no influence on the time of development of the tumors.

The carcinogenic activity of 2-acetaminofluorene. IV. Action of related compounds. R. H. Wilson, F. DeEds and A. J. Cox, Jr. Cancer Res. 7(7):453-458, July, 1947. The carcinogenic property of 2-acetaminofluorene has been demonstrated in rats and mice. The deacetylated compound, 2-aminofluorene, has also been shown to be effective in producing similar lesions in both species of animals when incorporated in the diet and fed to them for some time. Implanted crystals of aminofluorene were quite toxic, and in the single rat that

survived lesions were slight, perhaps because the compound was eliminated before malignant processes were well initiated.
Fluorene, 2-chlorofluorene, fluorenone, diethylaminoethyl-fluorene-9-carboxylate hydrochloride, and xanthone were not carcinogenic when administered to rats in the diet. It is suggested than an amino group in the 2-position is important in determining the carcinogenic activity of certain types of compounds.
Leukemia developed in a number of rats given propylene glycol subcutaneously by itself or as the solvent for the above compounds. This finding should be investigated further.

Effect of rutin on permeability of cutaneous capillaries. Anthony M. Ambrose and Floyd DeEds. Jour. Pharmacol. Exper. Therap. 90(4):359-363, August, 1947. The possibility of rutin protecting against capillary injury has been tested experimentally in rabbits using the accumulation of intravenously injected trypan blue into the irritated areas as a criterion of capillary damage. Capillary injury was produced by means of chloroform, intracutaneous injection of histamine, and the application of negative pressure. These procedures were applied to the depilated ventral surfaces of albino rabbits. Chloroform irritation was produced by the application for 30 seconds of a soaked cotton pledget. Each rabbit received previously 2 ml. of a 1% soln. of trypan blue intravenously. After control observations on the time of accumulation of dye into chloroform-irritated areas, the rabbits received intravenously 1 ml./kg. of a 20% soln. of rutin in propylene glycol.
In 22 rabbits, after the injection of rutin, the appearance of dye in newly formed chloroform wheals was delayed as much as 8 times over the control observations. Intracutaneous injection of histamine before and after rutin administration gave some indications that rutin protected against the local action of histamine. Five out of 6 rabbits to which negative pressure (30 mm. of Hg) was applied for 1 min. showed blue stained wheals immediately after release of suction before rutin injection, but none showed positive results when negative pressure was reapplied after rutin.
It is concluded that rutin protects rabbits against capillary damage under the conditions of our experiments.

The carcinogenic activity of 2-acetaminofluorene. Characteristics of the lesions in albino rats. A. J. Cox, Jr., R. H. Wilson and F. DeEds. Cancer Res. 7(10):647-657, Oct., 1947. Oral administration to rats of small quantities of 2-acetaminofluorene has been followed by the development of a wide variety of tumors in different tissues. This report presents further observations on the histological appearnace of these lesions, indicates a few additional types, and gives more information concerning the frequency of proliferative changes. The tissues reported on in this paper are from those rats which, because of the appearance of multiple lesions, had presumably received a thoroughly effective exposure to the compound. There were 84 such animals.
Most of the tumors are derived from epithelial cells. Most tissues which give rise to tumors are also the site of nodular epithelial hyperplasia which is not distinctly neoplastic. No sharp distinction can be made between these hyperplastic nodules and tumors formed by similar cells. Malignancy can be recognized in some of the tumors by the occurrence of tumor cell infiltration and metastasis. The factors which determine the localization of the experimental tumors are not known.